THE LIFE HISTORY OF POLYPODIUM POLYPODIOIDES HITCH., ESPECIALLY SPERMATOGENESIS

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A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

1933

Reprinted from the Journal of the
Elisha Mitchell Scientific Society
Vol. 49, No. 2, April, 1934

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Reprinted from the JOURNAL OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY
Vol. 49, No. 2, April, 1934

THE LIFE HISTORY OF POLYPODIUM POLYPODIOIDES HITCH., ESPECIALLY SPERMATOGENESIS[1]

By DOUGLASS E. RANKIN

PLATES 23–26

INTRODUCTION

The following study of the gametophyte of *Polypodium polypodioides* was carried out in search of a better understanding of the behavior of the nucleus, the plastids, and mitochondria during the several phases of development of this fern.

This work includes a general study of the development of the antheridium, of the archegonium, of fertilization, and of the development of the embryo. A cytological study of spermatogenesis, carried on not only with nuclear fixatives but also with cytoplasmic and mitochondrial ones, is likewise included.

HISTORICAL SURVEY

The structure and development of the antheridium and archegonium of the Leptosporangiate ferns have been extensively studied. A rather complete bibliography of the subject up to 1923 has been published by Bower (7) in his book on the Filicales and need not be repeated here. More recent work on these phases in certain Leptosporangiatae has been done by Miss Stokey (31) on the Cyathaceae and by Miss Hartman (16) on the opening of antheridia in certain Polypodiaceae.

Comparatively few workers have studied fertilization in the ferns. Studies have been published by Strasburger (32) on *Pteris* and *Ceratopteris*, by Campbell on *Pilularia* (9) and on *Osmunda* (10), by Shaw (29) on *Onoclea*, by Thom (34) on *Adiantum* and *Aspidium*, and by Yamanouchi (37) on *Nephrodium*.

As mentioned by Yamanouchi (37), the two features of especial interest recorded in the above papers are:

1. The presence of a wall around the egg after fertilization.
2. The frequently collapsed condition of the egg after fertilization.

Strasburger (32) has stated that more than one sperm is prevented

[1] Botanical Contribution from the Johns Hopkins University No. 126.

from entering the egg by the formation of a heavy enclosing wall about this immediately after fertilization by the first one. Shaw (29) on the other hand believes that a partial collapse of the egg cell occurs after one sperm has entered. In this condition additional sperms are not able to enter. Mottier (20) doubts that the above condition is normal.

The development of the embryo of the Leptosporangiatae has been studied by Atkinson (2), Campbell (10), Leitgeb (19), Shaw (29) and, more recently, by Cross (11). The occasional formation of more than one embryo on a prothallus was noted by Atkinson (2), Etter (14), and Mottier (22).

Strasburger (32 and 33), Belajeff (3, 4, 5, 6), and Shaw (30) made important studies of the spermatogenesis of ferns during the latter part of the nineteenth century. A question under debate with most of these workers was whether or not the blepharoplast is homologous with the centrosome. Strasburger (33), from his work on the zoospores and gametes of the algae and on the spermatozoids of ferns, concluded that the cilia on both arise from the blepharoplast and that these structures are morphologically distinct from centrosomes. Belajeff (5) was the earliest worker to describe the development of the blepharoplast in Pteridophytes. He first saw the cilia arising from the blepharoplast in the spermatozoids of *Equisetum*. The work of Belajeff (5) and Shaw (30) on the spermatogenesis of *Marsilea* led both to conclude that the blepharoplasts in that plant are identical with centrosomes. Webber (35) and Ikeno (18) worked on the spermatogenesis of the Cycadaceae. Webber believed the cilium-bearing organ to be quite distinct from the centrosome and it was he who named the former the "blepharoplast." Ikeno, however, believed the two organs to be identical.

In discussions of the morphological development of plant spermatozoids, the interest until recently has been centered in the metamorphosis of the nucleus and of the cilium-bearing organ. Of late, however, there has become evident an interest in certain cytoplasmic elements other than the blepharoplast; namely, the chondriosomes, the plastids, and the vacuoles. The part that these structures play in spermatogenesis is still a matter of uncertainty and of controversy.

Yamanouchi (37), in his study of the spermatogenesis of *Nephrodium molle*, found that the blepharoplasts are first discoverable at the resting stage before the last nuclear division in the antheridium. At this time they appear as two darkly staining bodies, one on either side of the nucleus. They remain near the pole of the spindle during the division but no asters are found around them. When the spermatids are formed

one of these bodies is seen in each cell some distance from the nucleus. At the same time there appears opposite the blepharoplast a smaller body which is called the Nebenkern. The blepharoplast applies itself to the nuclear membrane and elongates along with the nucleus, following half way around the two and a half times coiled spermatozoid. The Nebenkern remains in the cytoplasm at the side of the nucleus and is finally found in the vesicle attached to the lower end of the spermatozoid.

Certain of these same structures were found by R. F. Allen (1) in her work on spermatogenesis in ferns. She did not, however, discover blepharoplasts in the spermatid mother cell nor find a structure comparable to the Nebenkern. On the other hand she did, like Buller (8), find starch in the vesicle of the spermatozoid, a fact which earlier workers had noted but which more recent investigators had not mentioned.

In *Equisetum*, Sharp (27) found a single centrosome surrounded by astral radiations in the penultimate generation of the antheridium. This divides to form the blepharoplasts which pass, with their asters, to opposite sides of the nucleus and occupy the poles of the spindle. In the spermatid the blepharoplast becomes vacuolated and fragments. Later these parts unite to form the cilium-bearing band. This band becomes associated with the nucleus in forming the spermatozoid but is not so closely bound to it as is the case in the ferns. He also found a series of darkly-staining bodies along the concave surface of the nucleus, the origin or nature of which he did not determine.

In a study of the spermatogenesis of *Marsilea* also, Sharp (28) decided that the blepharoplast is of the nature of a centrosome. In the first mitosis of the spermatogenous tissue the two centrioles are weakly developed but show astral radiations. In the second, third, and fourth divisions, however, they are very distinct. The blepharoplast becomes vacuolate and fragments as in *Equisetum*, but to a less degree. The parts here also later unite to give rise to the band which produces the cilia.

So little work has been done in all plants on the cytoplasmic elements other than the blepharoplast that the discussion of the literature on these subjects will be more general in nature.

Gavaudan and Cazalis (15) studied the behavior of the plastids in the Characeae during spermatogenesis. They found that the plastids retain their normal appearance until the spermatid begins its transformation into the spermatozoid. The plastids are then said to form a granular band on the posterior surface of the spermatozoid.

Motte (20), from his study of spermatogenesis in the mosses, con-

cluded that both mitochondria and plastids are present in the early stages of the development of the antheridium. In the later stages of spermatogenesis the plastids lose their starch and fragment. These fragmented plastids plus the mitochondria condense to form the solid limosphere which is attached to the posterior portion of the spermatozoid nucleus.

Weier (36) traced the development of the plastid through spermatogenesis in *Polytrichum* and *Catharinaea*. The young antheridia of these mosses, according to Weier, possess many plastids but their number becomes reduced by successive cell divisions until each androgone has only one plastid. This divides prior to each of the next two divisions. During the last division the plastid becomes reticulate. In the androcyte this reticulate body condenses to form the limosphere. The limosphere buds off the apical body and the remainder forms the vesicle at the base of the sperm. The apical body comes in contact with the blepharoplast which draws it to the anterior part of the sperm where it forms a slender pointed cytoplasmic filament.

In his work on the origin and evolution of the plastids in Pteridophytes, Emberger (13) discusses the spermatogenesis of *Adiantum*. He finds plastids and mitochondria present in the antheridium initials. The plastids become smaller and finally in the mother cell of the spermatid are hardly distinguishable from the mitochondria. Vacuoles filled by phenolic compounds are found in the early stages of the antheridium and are always present in protoplasts of the wall cells. These disappear by the time the spermatid mother cells are formed. In the spermatids there appear vacuoles, the nature of the contents of which was not determined. The granules at this time are indistinguishable except for a slight difference in size. There is, however, as Emberger thinks, a separation of the granules in the subsequent development of the spermatozoid. The plastids migrate to the vesicle and the mitochondria form a double row of granules along the length of the spermatozoid.

Dracinschi (12) studied the mature sperms in several Eufilicineae, and in *Pilularia* of the Hydropteridineae. She discusses the structure of the sperm under the headings of the cilium-bearing band, the nuclear substance, and the plasma material. The cilium-bearing band and nucleus form the spiral body of the sperm. The cilium-bearing band is broad at the anterior end and tapers toward its base. It is somewhat more than half the length of the spermatozoid. It bears three rows of cilia on the anterior end and the number of rows decreases to one as the band becomes smaller. Each cilium possesses a small, expanded, basal

portion and a stiff shaft about two microns in length. The remainder of the cilium is flexible and fibre-like in character. A darkly-stained zone which she calls the *Randsaum* (or marginal hem) runs along the outer anterior edge of the sperm. This, according to Dracinschi, is the structure that Belajeff (5) thought gave rise to the cilia and is what Shaw (30) later mistakenly called the blepharoplast. The nucleus is covered with a thin cytoplasmic sheath. In this sheath, on the outer, posterior surface of the spermatozoid, are found plate-like bodies which Dracinschi thought to be Golgi material. Chromomeres are found in the nucleus. Adhering to the inner margins of the coils of the nucleus is a row of granules, the morphological nature of which is not known but which Dracinschi thought might be of either plastid or vacuolar nature.

Yuasa (38) also studied the mature sperms of Leptosporangiate ferns. He found that in *Pteris cretica* the cilium-bearing band and the marginal hem both run the whole length of the sperm.

MATERIALS AND METHODS

The prothallia used in the following experiments were obtained from spores of *Polypodium polypodioides* collected near Fayetteville, North Carolina. The spores were sown on powdered peat which had been sifted and boiled. The spores germinated in about ten days. Antheridia were formed after eight weeks and archegonia in approximately ten weeks.

Bouin's fixation was employed for fixing the prothallia used in the morphological study of the development of the sexual organs, of fertilization, and of the development of the embryo. The two most successful stains employed were Heidenhain's iron haematoxylin for the chromatin of the nuclei and Delafield's haematoxylin for the cell walls.

For the work on spermatogenesis various other fixing agents and stains were employed to bring out the different cell components. Flemming's weaker fixative followed by Heidenhain's haematoxylin was used for the blepharoplast and nucleus. Prothallia fixed in Bouin's mixture, after pre-fixation with ten per cent formalin neutralized with $MgCO_3$, gave striking pictures of the blepharoplast and also of the development and the various phases of structure of the nucleolus. The chromosomes were fixed by this technique, but the chromatin reticulum was not stained. Champy-Kull's fixative and Altmann's stain fixed and stained mitochondria, plastids, blepharoplasts and nucleoli. By this method the blepharoplast and nucleus of the mature spermatozoid were differ-

entiated; other combinations failed to show this. The mitochondria when fixed with Regaud's medium and stained with Heidenhain's haematoxylin were seen more distinctly than by any other method. The mitochondria in the mature spermatozoid were especially clear. Starch in the mature spermatozoid stained purple with gentian violet after Merkel's fixative. It was also stained in the spermatid and spermatozoid with Gram's iodine stain, following fixation by Regaud's method. Several other fixatives and stains were used but gave no essentially different results and hence will not be discussed here. Mann-Kopsch's fixing liquid showed the development of plastids; Bouin's fixative, with Feulgen's reaction as a stain, was used for the chromatin. Vital stains such as neutral red, methylene blue, and Janus green were employed also, but without satisfactory results.

MORPHOLOGICAL STUDY OF THE REPRODUCTIVE ORGANS, FERTILIZATION, AND THE DEVELOPMENT OF THE EMBRYO

Development of the prothallus. The first sex organs to appear on prothallia of *Polypodium polypodioides* are the antheridia. These occur on plants which are about two months old and are formed before the prothallium has become heart-shaped, or at least soon after the notch appears. The antheridium-bearing plants are small and one or more cells in thickness (figs. 1, 2). In some cases the antheridia cover practically the entire upper and lower surfaces of the prothallium, leaving only a narrow margin of sterile vegetative cells across the apical end. Such plants apparently never form archegonia. Other male plants which have not borne such a heavy crop of antheridia continue vegetative growth for some time and then form archegonia.

The first archegonia were formed on prothallia of the same sowing as that last mentioned which had *not* borne antheridia. They developed about two weeks after antheridia had appeared. The female plants were more characteristically heart-shaped and about three times as large as the male plants of the same culture. They possessed the typical archegonial cushion along the central portion of the prothallium. The archegonia are formed on this cushion from both the upper and lower surfaces. The archegonia form on the lower surface first, as is also true of antheridia. Only rarely were both types of reproductive organs found on the same plant at one time. When this did occur the antheridia were formed on proliferations from the margins or cushions of plants the bodies of which had previously borne no antheridia but archegonia only. A culture of *Polypodium polypodioides* about four months

old is shown in figure 3. This photograph shows the archegonial plants distinctly but the smaller, antheridial prothallia are not clear.

The development of the antheridium. The development of this organ resembles that found in many other Polypodiaceae. A prothallial cell enlarges and divides at an often slightly oblique angle, to give rise to a basal cell and an antheridium initial (fig. 42). The first division in the initial produces a cup-shaped cell whose rim touches the lateral wall of the antheridium initial and whose base may or may not come into contact with the upper wall of the basal cell (fig. 22). The second wall is concentric with the upper surface of the antheridium and intersects the first wall in a ring (fig. 22). In this upper cell is formed the third wall, a circular anticline, which cuts out a disk-like cover cell with sloping sides (fig. 23). It is this latter cell which, by bursting, or by being dislodged intact, allows the spermatozoids to escape. The three cells so formed surround a large central cell, which is the primary spermatogenous cell. In the central cell the first division is longitudinal and the second one transverse to the axis of the antheridium. After this one, two, or rarely three more divisions may occur, giving respectively 8, 16, and 32 spermatids. In some of the smaller prothallia none of the antheridia had more than eight spermatozoids. Generally, however, about half of the antheridia of the smaller plants produced eight spermatozoids each and half produced sixteen spermatozoids. On the larger prothallia, on the other hand, about one-third of the antheridia produced eight spermatozoids, and two-thirds produced sixteen. Sometimes a slightly larger proportion of the antheridia produced sixteen spermatozoids each, i.e. larger prothallia have larger antheridia. Antheridia with thirty-two spermatozoids were rare.

The mode of opening of the antheridium corresponds with that described by Miss Hartman for certain other Polypodiaceae. The cover cell, though it is occasionally extruded intact, is more often burst and its contents form a granular mass.

Development of the archegonium. The archegonia arise in acropetal succession on the cushion region of the prothallium. The initial cell of the archegonium is formed by a division of a superficial cell in this region (fig. 4). It can be identified by its large nucleus and by its cytoplasm which is denser than that of the surrounding vegetative cells.

When the archegonium initial is formed it undergoes two successive transverse divisions, giving rise to the basal cell, the central cell, and the primary neck cell (fig. 5). The central cell is, like the archegonium initial, conspicuous because of its large nucleus and dense cytoplasm.

The first division in the young archegonium is in the primary cover cell (fig. 6). The cell wall so formed is an anticline parallel to the axis of the archegonium. The two neck cells immediately undergo another anticlinal division perpendicular to the first. Thus are formed the four neck cells that are present at this time in the archegonium. The central cell, meanwhile, has started to enlarge. As it increases in size the neck cells divide by anticlines transverse to the neck and so keep pace with it, and in this manner is formed a neck which projects beyond the surface of the prothallus.

The first division in the central cell is transverse (fig. 7). This division gives rise to a large inner cell which is embedded in the vegetative tissue of the prothallus and a smaller primary neck canal cell that is surrounded by the wall cells of the neck which project beyond the prothallus. The inner or ventral cell divides before the primary neck cell. When the spindle for this division is formed it is situated in the outer portion of the cell (fig. 8). Thus when the wall is formed the two resulting cells are of quite unequal sizes. The larger inner one is the egg and the smaller outer one is the ventral canal cell. Following this division in the ventral cell a nuclear division takes place in the primary neck canal cell. This is also a transverse division. Most often a cleavage of the cytoplasm occurs, but no wall is formed between the two nuclei which are produced by this division (fig. 10). In some few cases, however, a wall may occur.

Thus the mature archegonium contains a large egg cell, a smaller ventral canal cell, and two neck canal cells or nuclei. As the archegonium matures the egg cell becomes indented due to pressure of the three canal cells above it (fig. 9). Then too, as the archegonium becomes older the two nuclei of the neck migrate to the outer end of the neck and become packed tightly together giving them, superficially, the appearance of being fused.

When prothallia bearing these mature archegonia are watered, the cells of the neck become more turgid and the inner cells swell. As a result the archegonium is ruptured at the apex. This leaves a chimney-like neck with an opening above, through which a slimy substance is discharged. The slimy substance is evidently a disintegration product of the three canal cells. The egg cell which was indented before the opening of the archegonium becomes rounded when pressure is released by the discharge of part of the contents of the neck.

Fertilization. The spermatozoids are attracted to the neck of the open archegonium. Pfeffer (26) and Hoyt (17) have noted that this

is due to the presence of malic acid or malates in the disorganized contents of the ventral canal and neck canal cells. Many spermatozoids were observed in the neck of the archegonium but never more than one could be found to have penetrated the egg (fig. 12). After fertilization eggs often presented a collapsed appearance, though other eggs, also containing spermatozoids and in approximately the same stage of development, were well rounded. No unusually heavy wall was observed around the recently fertilized eggs. The spermatozoid while in the neck of the archegonium is very much elongated and loosely coiled (fig. 11). When, however, it has entered the egg the spermatozoid condenses to form a thick, coiled body (fig. 12). The chromatin of the egg nucleus during the penetration of the sperm forms a coarse net. The sperm nucleus next becomes reticulate (fig. 13). Soon the reticulum of the male nucleus becomes indistinguishable from the reticulum of the egg nucleus.

Development of the embryo. The fertilized egg enlarges greatly during the resting stage preceding the first division (fig. 14). The first wall formed is slightly oblique to the neck of the archegonium and transverse to the long axis of the prothallus (fig. 15). This divides the embryo into anterior and posterior halves. The second wall is transverse to the neck of the archegonium and parallel to the long axis of the prothallus (fig. 16). The embryo is thus divided into quadrants. The next wall is the octant wall which is parallel to the neck of the archegonium and in the plane of the long axis of the prothallus. Two each of these octants are used in the formation of the primary organs of the plant. In an embryo developed on the lower surface of the prothallus the cotyledon is formed in the lower anterior segments, the stem from an upper anterior segment, the root from a lower posterior segment and the foot from the upper posterior segments. The walls developed within the octants are somewhat irregular. The first walls, however, are nearly always parallel to the anticlinal octant walls (figs. 17 and 18).

The initials are distinguishable even in embryos as young as that shown in figure 18. An older stage showing the root, stem, and cotyledon initials is illustrated in figure 19. In this embryo the quadrant walls are also distinct. A still older embryo in which the root and cotyledon have elongated is shown in figure 21.

CYTOLOGICAL STUDY OF SPERMATOGENESIS—STRUCTURE OF THE SPERMATID AND THE SPERMATOZOID AS DETERMINED BY THE USE OF SEVERAL CYTOLOGICAL METHODS

1. Results with Flemming's fixative and Heidenhain's haematoxylin stain

In material fixed and stained by this combination of methods the chromatin and the blepharoplasts are dark blue. This is an excellent fixative and stain for these elements and their development can easily be demonstrated with it. The central cell has a large nucleus and one or two large and often irregular nucleoli (fig. 22). With this technique the nucleoli are distinguishable up to, and sometimes after, the development of the spermatids but never show as distinctly as with the two methods to be described next.

The blepharoplasts are first discoverable in the resting stage of the spermatid mother cell (fig. 24). They appear as two darkly staining bodies, one lying on each side of the nucleus at some distance away from the wall of the latter. The chromatin of the nucleus then forms a coarse reticulum with distinctly thicker and thinner portions. At prophase the blepharoplasts move still farther away from the nucleus and occupy the poles of the spindle, but show no astral radiations (fig. 25). The metaphase, telophase, and anaphase were not found in cells fixed and stained by this method. Cells fixed by Champy-Kull's method showed the blepharoplast during the metaphase. During the resting stage of the spermatid nucleus the blepharoplast appears as a very conspicuous black body lying in the cytoplasm at some distance from the nucleus and the chromatin of the latter appears as a dense network (fig. 26). The blepharoplast approaches the nucleus and finally becomes applied to the nuclear membrane (fig. 27). At this stage it begins to elongate (fig. 28). When the blepharoplast has extended a little over half way around the still globular nucleus, the latter begins to change its form and inner structure. The threads of the chromatin network become more and more thickened (fig. 29) while the side of the nucleus opposite the blepharoplast becomes at first flattened and finally concave (fig. 30). During the time the nucleus is thus crescent shaped in lateral view the blepharoplast *cannot be differentiated with haematoxylin*. When the chromatin has condensed to form the more attenuate, coiled spermatozoid nucleus, however, the blepharoplast can be shown, extending approximately one coil beyond the nucleus, which itself shows from one and one-fourth to one and one-half turns (fig. 31).

2. Results obtained with neutral formalin, Bouin's fixative, and Heidenhain's haematoxylin stain

This method fixes and stains nucleoli, blepharoplasts, and the chromosomes. It does not stain chromatin when in the reticulum stage. The central cell shows one or two large, and often irregular, nucleoli (fig. 33). In the two-celled stage some cells were found with one nucleolus, some with two nucleoli and some with nucleoli in the process of division by constriction. Nucleoli in one or the other of these three phases were found in the resting nuclei of all stages up to the time the spermatid nucleus began its metamorphosis into a spermatozoid. The following tables show the combinations of those cell stages and nucleolar phases that were actually found:

(1) Four-celled stage.
  (a) Each of the four cells of the antheridium contained one nucleolus.
  (b) Each of the four cells of the antheridium contained two nucleoli.
  (c) Three cells of the antheridium contained two nucleoli and one had a dividing nucleolus.
(2) Eight-celled stage.
  (a) Each of the eight cells of the antheridium contained one nucleolus.
  (b) Five of the eight cells of the antheridium contained two nucleoli and three cells contained one nucleolus each.
  (c) One cell of the antheridium contained two nucleoli and seven cells contained one nucleolus each.
(3) Sixteen-celled stage.
  (a) Each of the sixteen cells of the antheridium contained one nucleolus only.
  (b) Each of the sixteen cells of the antheridium contained two nucleoli.

This technique makes the blepharoplasts even more conspicuous than does Flemming's, and the same developmental stages of these were found.

3. Results with Champy-Kull's fixative and Altmann's stain

These fix and stain blepharoplasts, mitochondria, plastids, and nucleoli distinctly, but stain chromosomes only faintly. In addition to the nucleus and the plastids this method demonstrates the presence in the prothallial cells of certain small purple-staining granules. In the prothallial cell at the mitotic division preceding the formation of the antheridium initial the mitochondrial granules, which have increased in number, are grouped about the poles of the spindle (fig. 42). Thus, when the initial of the antheridium is formed, it contains the plastids

which were in the outer part of the prothallial cell and the mitochondrial granules which surrounded the daughter nucleus. In the wall cells there are numerous plastids, smaller in size than those of the prothallial cells. The mitochondria in the wall cells are more numerous than in the prothallial cells but not as abundant as in the spermatogenous cells. These latter cells, on the contrary, though they have many more mitochondria, have fewer and smaller plastids than the wall cells (fig. 43). The plastids in both can be recognized by their deeply-staining outer portion and lightly-staining central region. The cells resulting from the divisions up to the spermatid mother cell stage are essentially alike. The mitochondria are very numerous and grouped about the poles during mitosis. The plastids up to this spermatid mother cell stage are still easily distinguishable. The one or two nucleoli of each cell stain faintly.

With this technique the blepharoplasts appear at metaphase of the division of the spermatid mother cell, as two conspicuous red bodies in each cell (fig. 46). The spermatid at its formation contains numerous mitochondria scattered through the cell (fig. 47). It also contains a large blepharoplast located at some distance from the nuclear membrane. The development of the blepharoplast has been described above. With other techniques, however, it was not distinguishable in the mature spermatozoid. With Champy-Kull's method the red stained blepharoplast can be seen to extend beyond the purple stained nucleus and is applied to a relatively small part of the upper portion of the nucleus (figs. 50, 51). As the nucleus begins to coil, preparatory to forming the spermatozoid, the mitochondria seem to become more closely associated with it (fig. 49). In the mature spermatozoid they form a double row of granules on the inner surface of the coiled nucleus (figs. 50, 51). This condition will be discussed in greater detail when recording the results obtained with Regaud's technique. The plastids were not distinguishable with the Champy-Kull method after the nucleus began to coil.

4. Results with Regaud's fixative and Heidenhain's haematoxylin stain

This method fixes and stains both mitochondria and plastids. Mitochondria fixed by this technique are vastly different in appearance from those fixed with Champy-Kull's. Fixed with Regaud's fluid and stained with Altmann's stain they appear as short rods and as when fixed with Champy-Kull are far more numerous in the central cell than in the wall cells. The plastids, which are about equal in length to the mitochondria, are distinguishable from them by their heavily staining outer portion

and lightly-staining interior. The rod-like type of mitochondrium is present up to the formation of the spermatid. In the spermatid the mitochondria appear either as short rods (fig. 54) or as longer rods forming a more or less broken chain (fig. 58). At this stage the plastids also are still distinguishable (fig. 56). As the nucleus begins to coil, the rod-like mitochondria are found along its edges and scattered over its surface (figs. 59, 60). As the nucleus coils more and more the mitochondria become arranged in two rows, like two strings of beads, one along each edge of the nucleus on the inner surface of the coil and then extend to form a single row along that portion of the blepharoplast which protrudes beyond the nucleus (figs. 61, 62, 63).

DISCUSSION

Morphological study of Polypodium polypodioides

In studying the development of the prothallus of *Polypodium polypodioides* several problems of interest arose. It was found that antheridia and archegonia are produced in almost equal abundance on the upper as well as the lower surface of their respective prothallia. Reproductive organs appear first on the lower surface but soon form on the upper surface also. Another point of interest was the fact that archegonial plants very commonly formed proliferations from the margins and cushion of the prothallia. Mottier (23) has described these from cultures which he kept growing for many years. In *Polypodium polypodioides* they occurred on prothallia which were about six months old and which had apparently been exposed to no adverse conditions.

In the development of the archegonium it was found that sometimes a wall is formed between the two nuclei of the neck canal cell. This was noticed by Campbell (10) also in *Osmunda*. This condition in both *Osmunda* and *Polypodium polypodioides* is a rare occurrence.

The factor which prevents more than one sperm from entering the egg is as yet undetermined. Shaw (29) thinks that it is accomplished by the collapse of the egg after the sperm has entered. Mottier (21), on the other hand, thinks this collapsed condition is due to fixation. Strasburger (32) said that a heavy wall formed around the egg immediately after fertilization, and that this prevented additional sperms from entering. It was observed in *Polypodium polypodioides* that a few eggs which contained spermatozoids were collapsed. Many other eggs containing spermatozoids, in apparently the same stage, were turgid. Two conclusions might be drawn from this. Either the collapsed state of the recently fertilized egg is due to fixation or it is a normal process. If

it is normal the rounded fertilized eggs are older than the collapsed ones and have passed through that phase and later have regained their turgor. No unusually heavy wall was ever found around these eggs. Thus more evidence was obtained for Shaw's view than for the view held by Strasburger.

The development of the embryo of *Polypodium polypodioides* is much like that of other Polypodiaceae. Polyembryony, probably due to cultural conditions, was often found. As many as eight young embryos were sometimes found on a single plant and as many as three embryos on one plant might have one cauline leaf developed. This condition has been noted by Mottier (22) in certain Polypodiaceae and Osmundaceae.

CYTOLOGICAL STUDY

The nucleus and the blepharoplasts, as stated above in the review of the literature, are the objects which have received most attention in recent studies of spermatogenesis. The investigations of Belajeff (5, 6) and Shaw (30) on spermatogenesis in *Marsilea* and of Sharp (27) in *Equisetum* led them to conclude that the blepharoplasts in plant spermatozoids are morphologically the same as the centrosomes. They have also shown that the cilia arise from the blepharoplast. The work of Yamanouchi (37) on spermatogenesis in *Nephrodium* shows that the blepharoplast in that fern is lacking many of the characters of a centrosome. It does not divide to form daughter centrosomes, it only occasionally occupies the poles of the spindle and does not have astral radiations formed about it.

The blepharoplasts in *Polypodium polypodioides* first appear at the resting stage prior to the last division in the antheridium (fig. 24) as darkly-staining bodies, one on either side of the nucleus. During the last division they are found to occupy the poles of the spindle but no astral radiations are seen around them (fig. 25). When this division is completed and the daughter cells are formed there is found in each spermatid one large blepharoplast, situated in the cytoplasm at some distance from the nucleus (fig. 26). The chromatin of the spermatid nucleus at this time forms a coarse reticulum. The blepharoplast next approaches the nuclear membrane and finally comes in contact with it (fig. 27). After it has become applied to the nuclear membrane the blepharoplast begins to elongate. In the early stages of its elongation one end of the blepharoplast may be in contact with the nucleus while the other end extends out into the cytoplasm (fig. 28). As it elongates further, however, the blepharoplast extends half way around the still

globular spermatid nucleus. In preparations which show the coarse chromatin reticulum no nucleoli are seen. Other fixatives, however, brought out the fact that there are present in the spermatid one or two large nucleoli which are conspicuous until the nucleus begins to coil. After the blepharoplast has reached half way around the spermatid nucleus the chromatin begins to condense. The nucleus begins to flatten on the side opposite the blepharoplast and finally becomes concave (fig. 30). The chromatin continues to condense until it forms a solid, strap-like coiled body. The blepharoplast is applied to about one-third of the upper portion of the nucleus and extends one coil beyond the nucleus (fig. 31). This development of the blepharoplast and nucleus resembles that found in *Nephrodium* by Yamanouchi (37). Yamanouchi, however, does not find the blepharoplasts constantly located at the spindle poles during the last division, while in *Polypodium polypodioides* this does seem always to be true. Thus it appears that the blepharoplasts in this plant retain one centrosome-like character. The blepharoplast in this fern, as was also true in *Nephrodium*, is always a solid body. No segmentation occurs such as was found by Sharp (28) in *Marsilea* and *Equisetum* (27). The connections of the cilia with the blepharoplast were not seen nor could the writer find any division of the blepharoplast into a marginal hem and a cilium-bearing band such as Miss Dracinschi (12) describes.

The nucleolus. The changes in the nucleolus during spermatogenesis have not been discussed very extensively in most earlier studies of this stage. This is due to the fact that nucleoli are usually not very conspicuous in material fixed primarily for the study of chromatin. Yamanouchi (37) found either one or two nucleoli present in the spermatid of *Nephrodium*. And Sharp (27) in *Equisetum* figures from one to three nucleoli in the spermatogenous cells before their last division and but one nucleolus in the spermatid.

In *Polypodium polypodioides* the nucleoli stained very clearly with Heidenhain's haematoxylin after fixation with neutral formalin followed by Bouin's mixture. The central cell of the antheridium may contain one or two large, irregular nucleoli (fig. 33). In subsequent stages the nucleoli are smaller and more rounded. All nuclei in cells ranging from those in the two-celled antheridia to those of the spermatid contain either one nucleolus, a nucleolus in the process of constriction, or two nucleoli. For example, one four-celled antheridium was found in which three cells contained two nucleoli and one cell contained a nucleolus in the process of constriction. One eight-celled antheridium

was found with five nuclei containing two nucleoli and three nuclei containing one nucleolus each. Where there were two nucleoli in a nucleus these nucleoli were much smaller than that of a nucleus which had but one nucleolus. These facts give clear evidence that the nucleolar material in the antheridium of *Polypodium polypodioides* divides prior to nuclear division. The relation of the nucleolus to the chromatin reticulum has not thus far been studied since the neutral formalin rendered the chromatin unstainable. No trace of the nucleolus during mitosis was found.

Development of the plastids. In considering the development of the plastids in ferns it was thought advisable to review that phase of spermatogenesis in some other plants.

Gavaudan and Cazalis (15) studied the plastids in the spermatogenesis of the Characeae and found that the plastids containing carotin and starch stain distinctly up to the time that the spermatid nucleus begins to coil. At the time of the formation of the spermatozoid the plastids form a granular band on its posterior surface. A granular band was also seen in *Chara* by Muhldorf (25) who, however, found it to be composed only of starch.

Motte (20), working on spermatogenesis in the mosses, found a chondriome made up of long, narrow plastids and smaller mitochondria in the early stages of the antheridium. The plastids lose their starch and fragment to form, with the existing mitochondria, a granular mass which condenses to form a solid limosphere. In *Polytrichum*, Weier (36) found that one plastid remained in the androcyte, became reticulate and finally formed the limosphere. Then the limosphere, by division, formed the limosphere remnant and the apical body. The former contains starch and is found at the base of the spermatozoid; the latter is applied to the blepharoplast.

This work on the development of the plastids during spermatogenesis in the Characeae and in the mosses serves to emphasize several points. First, the behavior of the plastids during spermatogenesis varies in different groups. Second, the plastids in these groups have a complicated but regular developmental history during this process. Third, the plastids furnish the mature spermatozoid with the starch which is necessary to its functioning.

Yamanouchi (37), in his work on *Nephrodium*, did not discuss the early development of the antheridium. He does state, however, that the cytoplasm of the spermatid mother cell does not generally contain plastids. This was due, probably, to the fact that he used Flemming's

fixative which does not satisfactorily fix plastids except when they are mature.

Sharp (27), in his study of *Equisetum*, found plastids in various stages of disorganization up to the 8- or 16-celled stage and even in the spermatid. His interest at this time was, however, centered in the blepharoplast, hence in order to see these clearly he selected cells that contained the fewest plastids. The present work on *Polypodium polypodioides* does not indicate that the plastids disorganize but rather that they are present in all stages of spermatogenesis. The presence of these plastids could always be determined by their characteristic appearance during the early stages or in later stages by their contained starch.

Emberger (13) used Regaud's fixative in his study of spermatogenesis in ferns. He found that the wall cells decrease in size from losing their starch. The plastids of the central cell of the archegonium decrease in size until they are but slightly larger than the mitochondria. They can, however, be distinguished from the mitochondria by this slight excess of bulk. After the last division they form very small granules, practically indistinguishable from the mitochondria. A part of the granules migrate to the protoplasmic vesicle in the coil of the spermatozoid. He concludes that these bodies are the plastids since earlier workers, for example, Buller (8), found starch present in these vesicles. Recently, Muhldorf (24) also found starch in the protoplasmic vesicles of fern spermatozoids.

The plastids in the prothallia of *Polypodium polypodioides* are of the type described by Zirkle (39) for the higher plants. They are hollow, flattened spheroids. These plastids cover completely the exposed surfaces of the prothallial cells. They are composed of an outer chromatic stroma and an internal vacuole in which starch is stored. The plastids stain pink with Altmann's stain used after fixation with Champy-Kull's mixture. With this fixative and stain their development can be traced through all stages from the prothallial cells to the spermatid.

When the initial cells of the antheridium are cut off from the prothallial cells the plastids, which are found in the initials, are slightly smaller than those of the vegetative cells. The two divisions which follow in the initial cell divide that cell into an upper and lower wall cell and a central cell which is the primary spermatogenous cell (fig. 43). In the two wall cells the plastids are approximately of the same size as those of the antheridium initial. The plastids in the central cell on the other hand have decreased in size since they fail to grow after each division. They still possess, however, their characteristic structure, an outer

chromatic layer and an internal vacuole. Although the plastids vary in size, shape, and number in individual cells they are essentially alike in all cells of the spermatogenous tissue from the central cell to the formation of the spermatid (figs. 43–45).

After the spermatids are formed the plastids are no longer distinguishable in material fixed with Champy-Kull's fixative and stained with Altmann's stain. It was thus necessary to devise some method by which the presence of plastids in the spermatids could be detected. It was found after many unsuccessful attempts that material fixed in Regaud's mixture and stained by Gram's iodine method showed the presence of starch in the spermatid and in the spermatozoid. The starch was not noticeable in the younger stages of the antheridium with this method. It was then assumed that the starch had increased greatly in amount in the spermatid. These large starch grains in the plastids stretched the walls to such an extent that they could not be distinguished even after staining. Starch was also found in the spermatozoid when material fixed in Merkle's fixative was stained with Flemming's triple stain (fig. 52). With this method the starch in both the spermatozoid and the prothallial cells stained purple. The starch present in the spermatozoid is contained in the protoplasmic vesicle at the base of the spermatozoid.

From this work on the behavior of the plastids during spermatogenesis in *Polypodium polypodioides* it was found that the plastids play a definite rôle during this process. They can be identified in all cells before spermatid formation by size, structure, and staining reaction. After the spermatids are formed they can be identified by the starch which they contain.

The chondriome. There has been very little mention of the chondriome in connection with the discussion of spermatogenesis in plants, although it has long been known to be an important element in animal spermatogenesis.

In the mosses Motte (20) found that the mitochondria, and the fragmented plastids condense to form the limosphere. Weier (36) did not find any evidence of the chondriome in *Polytrichum*. He thought this might be due to some uncontrolled factor in the technique employed.

The only discussion of the behavior of the chondriome as such, during spermatogenesis in ferns, is that of Emberger (13). He finds that a few mitochondria are present in the antheridial initial and a larger number are present in the spermatogenous cells. When the nucleus of the spermatid begins to coil these granules are visible in the cytoplasm

surrounding it. In the mature coiled spermatozoid they are seen as two rows of granules along the inner surface of the coil. The mitochondria found in *Polypodium polypodioides* have essentially the same development and resulting position as that described by Emberger (13). There are points of variation which will be discussed later but these do not seriously interfere with the interpretation.

Two other workers, Sharp (27) and Miss Dracinschi (12), have described similar bodies in the mature spermatozoid. Sharp found a row of dark, rounded, bodies ranging, in *Equisetum*, along the inner surface of the coiled spermatozoid. He has mentioned them only to state that their nature and origin were not determined. Bodies of a similar appearance were also seen in the spermatozoid of the Eufilicinae and one of the Hydropteridineae, *Pilularia*, by Miss Dracinschi. She also could not explain their nature. The mitochondria in *Polypodium polypodioides* appear to be very similar to those described by Emberger (13) and those figured by Dracinschi (12) and by Sharp (27).

The mitochondria in *Polypodium polypodioides*, as has been stated above, differ somewhat from those of *Adiantum* described by Emberger (13). This difference is a matter of inconstancy in their shape, and in their size and arrangement at certain stages.

With Champy-Kull's fixative and Altmann's stain a few mitochondria are found to be present in the prothallial cells. They are short rods or granules which stain violet. They are found sometimes enveloping the plastids and sometimes in the cytoplasm between the plastids. During the division of the prothallial cell which forms the antheridium initial the mitochondria are found grouped about the spindle poles (fig. 42). In the initial cell of the antheridium the granules are more numerous than in the prothallial cells. The mitochondria are still more numerous in the wall cells of the antheridium than in the initial cells. However, they are fewer in number than in the central or primary spermatogenous cell. In this central cell there are exceedingly numerous purple granules, much smaller than the plastids, which are also present at that time (fig. 43). The mitochondria in all cells up to the formation of the spermatid have practically the same appearance as those of the central cell. The mitochondria are grouped about the poles of the spindle during the mitotic divisions of the spermatogenous tissue as was also true in the divisions which formed the antheridium initial.

When the spermatids are formed mitochondria are still abundant and scattered throughout the cytoplasm of the cell. The mitochondrial

granules begin to migrate toward the nucleus from the outer portions of the cytoplasm when the nucleus of the spermatid begins its metamorphosis. They can be observed scattered over its outer surface when the nucleus is partially coiled; however, they are usually more numerous along the margins of the coil (fig. 49). By the time the chromatin of the nucleus has condensed to form a nearly solid body the mitochondria are found as a double row of granules along the inner surface (figs. 50, 51).

Regaud's fixative gave two quite different results. In some material the mitochondria appeared as short rods in the prothallial cells, all the antheridial cells, and spermatids (fig. 54). The mitochondria grouped themselves about the metamorphosing nucleus and finally migrated to the under surface where they formed the two rows of granules. In other prothallia fixed with Regaud's fixative the mitochondria were seen to form a broken network in the antheridium initial instead of forming separate rods (fig. 53). This network was observed also in the different stages of the antheridium up to the last division of the spermatogenous tissue. In the spermatid this network was especially noticeable and while the nucleus is central these mitochondria seem to surround the nucleus on all sides (figs. 57, 58).

After the nucleus has condensed as in the preceding cases, the mitochondria form the double row of granules around the inner surface of the spermatozoid (figs. 61–63). Between the rod-like mitochondria and the mitochondria which formed the broken network there were many intermediate stages. It was thus concluded that there was either some slight variation in technique during different fixations or that the physiological condition of the prothallia varied. Either, it is believed, could account for a difference in the morphological character of the mitochondria.

The differences in the mitochondria as shown by Champy-Kull's and by Regaud's fixatives must be due to technique. The fact that in both cases the granules form a double row along the inner surface of the spermatozoid is proof of the fact that these are identical structures.

SUMMARY

Morphological. Antheridia appear on prothallia of *Polypodium polypodioides* when the plants are about two months old. Archegonia appear about two weeks later on other plants of the same sowing which are approximately three times as large as the male plants mentioned. Both types of organs occur on both upper and lower surfaces of the

prothallia. Antheridia and archegonia occurred on the same plants simultaneously only where antheridia arose on proliferations of plants that had already borne archegonia.

The development of the antheridium and archegonium corresponds to that in many other Polypodiaceae. The opening of the antheridium is by the extrusion of the cover cell with wall intact or as a granular mass. In the archegonium, a wall is occasionally formed separating the two nuclei of the neck canal cells.

The ripe antheridia and archegonia open in a few minutes after the prothallia are watered. Many spermatozoids enter the open neck of the archegonium, but only one penetrates the egg. The egg is turgid before the penetration of the sperm. Many of the eggs observed immediately after fertilization were in a collapsed condition. No especially heavy wall was formed around the recently fertilized eggs.

Cytological. The blepharoplasts arise in the resting stage of the spermagones before the last nuclear division in the antheridium. They occupy the poles of the spindle during the subsequent mitosis. When the spermatids are formed one blepharoplast is found in each cell and is situated at some distance from the nucleus. The blepharoplast moves toward the coarsely reticulate nucleus and becomes applied to the nuclear membrane. Here it elongates until it has reached one-half way around the still globular spermatid nucleus. At this time the nucleus begins to condense and forms a strap-like coiled body. The blepharoplast, in a mature spermatozoid which is still inside the antheridium, has stretched to form about four-fifths of a complete coil and the nucleus forms about one and a half coils. The blepharoplast extends beyond the nucleus approximately half of a coil.

The structure and development of the nucleoli was traced, using material fixed in neutral formalin followed by Bouin's fluid. One or two large irregular nucleoli are found in the central cell of the antheridium. One or two nucleoli are also almost constantly found during the subsequent generations of the spermatogenous tissue. Oftentimes a constricted nucleolus was observed. These latter were seen even after the spermatid was formed and the blepharoplasts had begun their elongation. Where there are two nucleoli in a nucleus the nucleoli are much smaller than the single nucleolus which is sometimes found in each nucleus. The presence of sometimes one nucleolus, of two nucleoli, or in other cases of a constricting nucleolus, and the relative sizes of the nucleoli, all indicate that the nucleolus divides prior to each nuclear division.

The plastids were identified by their morphological characteristics in the prothallial cells, the antheridium initial, the wall cells, and in all spermatogenous cells until the spermatids were formed. After the formation of the spermatids the plastids were identified by their contained starch. This starch is abundant in the spermatids and mature spermatozoids. No starch was found in younger stages. The plastids of the spermatids were stretched to such an extent by enclosed starch that their boundaries could not be seen, even after staining.

A few rod-like or granular mitochondria are found in each prothallial cell. They are either grouped around the plastids or are scattered in the cytoplasm. In the division prior to the formation of the antheridium initial they are grouped about the poles of the spindle. The mitochondria are found in the antheridium initial and the antheridial walls, but are not as numerous there as in the spermatogenous tissue.

The mitochondria appear as short rods or granules with Champy-Kull's fixative and Altmann's stain. With Regaud's fixative they appear as long rods in some fixations and in other fixations have the appearance of a broken network. The mitochondria are very numerous in all stages of the spermatogenous tissue when both Champy-Kull's and Regaud's fixatives are used.

Soon after the nucleus begins to condense the mitochondria migrate from the peripheral cytoplasm toward the nucleus. They form a sheath around the partially condensed nucleus. The granules continue their migration as the spermatozoid matures. And in the fully developed spermatozoid they are found as two beaded rows along the inner surface of the coiled nucleus and extending as one row along that portion of the blepharoplast which protrudes beyond the nucleus.

It is a pleasure for the author at this time to acknowledge her indebtedness to Professor Duncan S. Johnson who suggested this problem and under whose direction this work was carried on. She would like also to thank Professor Conway Zirkle of Philadelphia for suggesting certain fixatives used and Professor Ralph E. Cleland of Baltimore for very helpful advice.

BIBLIOGRAPHY

(1) ALLEN, RUTH F. 1911. Studies in spermatogenesis and apogamy in ferns. Trans. Wisconsin Acad. Sci., Arts and Letters **17** (1): 1–56.

(2) ATKINSON, G. F. 1894. The study of the biology of ferns by the collodion method, pp. 23–44.

(3) BELAJEFF, W. 1889. Mittheilungen über Bau und Endwicklung der Spermatozoiden bei den Gefässkryptogamen. Ber. deutsch. bot. Ges. **7**: 122–125.
(4) ———. 1897. Ueber den Nebenkern in spermatogenen Zellen und die Spermatogenese bei den Farnkräutern. Ber. deutsch. bot. Ges. **15**: 337–339.
(5) ———. 1898. Ueber die Cilienbildner in den spermatogenen Zellen. Ber. deutsch. bot. Ges. **16**: 140–144.
(6) ———. 1899. Ueber die Centrosome in den spermatogenen Zellen. Ber. deutsch. bot. Ges. **17**: 199–205.
(7) BOWER, F. O. 1923. The Ferns (Filicales), vol. 1. Cambridge, at the University Press.
(8) BULLER, A. H. R. 1900. Contributions to our knowledge of the physiology of the spermatozoa of ferns. Ann. Bot. **14**: 543–582.
(9) CAMPBELL, D. H. 1888. The development of *Pilularia globulifera* L. Ann. Bot. **2**: 233–264.
(10) ———. 1892. On the prothallium and embryo of *Osmunda claytoniana* L. and *O. cinnamomea* L. Ann. Bot. **6**: 49–94.
(11) CROSS, G. L. 1931. Embryology of *Osmunda cinnamomea*. Bot. Gaz. **92**: 210–217.
(12) DRACINSCHI, M. 1930. Über das reife Spermium der Filicales und von *Pilularia globulifera*. Ber. deutsch. bot. Ges. **48**: 295–311.
(13) EMBERGER, L. 1921. Recherches sur l'origine et l'evolution des plastides chez les Pteridophytes. Arch. Morph. gen. et exp. **1**: 1–190.
(14) ETTER, A. 1923. Polyembryony developed under experimental conditions in certain polypodiaceous ferns. Bull. Torrey Bot. Club **50**: 95–107.
(15) GAVAUDAN, P., AND R. CAZALAS. 1931. Nouvelles observations sur la spermatogénèse des Characées. Nature de la bande granuleuse et apparition de carotène et de chlorophylle dans certains spermatocytes. Compt. Rend. **193**: 193–195.
(16) HARTMAN, M. E. 1931. Antheridial dehiscence in the Polypodiaceae. Bot. Gaz. **91**: 252–276.
(17) HOYT, W. 1910. Physiological aspects of fertilization and hydridization in ferns. Bot. Gaz. **49**: 340–370.
(18) IKENO, S. 1898. Untersuchungen über die Entwickelung der Geschlechtsorgane und den Vorgang der Befruchtungung bei *Cycas revoluta*. Jahrb. Wissen. Bot. **32**: 557–602.
(19) LEITGEB, H. 1878. Zur embryologie der farne. Zitzungsber. d. Mathem Klasse d. R. Akad. Wiss. zu Wien **75**.
(20) MOTTE, J. 1928. Contribution a la connaissance cytologique des Muscinées. Ann. Sci. Nat., Bot. X^e serie, **10**: 293–543.
(21) MOTTIER, D. M. 1904. Fecundation in plants. Carnegie Inst., Washington.
(22) ———. 1925. Polyembryony in certain Polypodiaceae and Osmundaceae. Bot. Gaz. **80**: 331–336.
(23) ———. 1931. Development of sex organs of fern prothallia under prolonged cultivation. Bot. Gaz. **92**: 218–223.
(24) MUHLDORF, A. 1930. Über die Starke in pflanzlicher Spermien. Bot. Arch. **30**: 167–194.

(25) ———. 1930. Berichtigungen und Ergänzungen unserer Kenntnisse über die Morphologie und Histologie pflanzlicher Spermien. Biol. Gen. **6**: 457–482.
(26) PFEFFER, W. 1884. Locomotorische Richtungsbewegungen durch chemische Reize Untersuch. aus dem bot. Inst. zu Tubingen **1**: 363–482.
(27) SHARP, LESTER W. 1912. Spermatogenesis in *Equisetum*. Bot. Gaz. **54**: 89–119.
(28) ———. 1914. Spermatogenesis in *Marsilea*. Bot. Gaz. **58**: 419–431.
(29) SHAW, W. R. 1898. The fertilization of *Onoclea*. Ann. Bot. **12**: 261–285.
(30) ———. 1898. Ueber die Blepharoplasten bei *Onoclea* und *Marsilea*. Ber. deutsch. bot. Ges. **16**: 177–184.
(31) STOKEY, ALMA G. 1930. Prothallia of the Cyatheaceae. Bot. Gaz. **90**: 1–45.
(32) STRASBURGER, E. 1869. Die Befruchtung bei den Farnkräutern. Jahrb. wiss. Bot. **7**: 390–408.
(33) ———. 1892. Schwärmsporen, Gameten, pflanzliche Spermatozoiden und das Wesen der Befruchtung. Histol. Beitr. **4**: 47–158.
(34) THOM, C. 1899. The process of fertilization in *Aspidium* and *Adiantum*. Trans. Acad. Sci. St. Louis **9**: 285–314.
(35) WEBBER, H. J. 1901. Spermatogenesis and Fecundation of *Zamia*. U. S. Dept. Agric., Bur. Plant Ind., Bull. **2**: 1–100.
(36) WEIER, T. ELLIOT. 1931. A study of the moss plastid after fixation by mitochondrial, osmium and silver techniques. II. The plastid during Spermatogenesis in *Polytrichum commune* and *Catharinaea undulata*. La Cellule **41**: 51–84.
(37) YAMANOUCHI, S. 1908. Spermatogenesis, oogenesis, and fertilization in *Nephrodium*. Bot. Gaz. **45**: 145–175.
(38) YUASA, AKIRA. 1932. Studies in the cytology of Pteridophyta. I. On the spermatozoid of *Pteris cretica* L., var. *albolineata* Hk. Bot. Mag. (Tokyo) **46**: 4–12.
(39) ZIRKLE, C. 1926. The structure of the chloroplast in certain higher plants. Amer. Jour. Bot. **13**: 301–341.

DESCRIPTION OF PLATES

Figures 1–3 are photographs of living prothallia. Figures 4–63 are from paraffin sections of fixed prothallia. Figures 22–32 were fixed with Flemming's fixative and stained with Heidenhain's haematoxylin. Figures 33–40 were fixed with neutral formalin and Bouin's fluid and stained with Heidenhain's haematoxylin. Walls of cells and chromatin reticulum not stained. Figures 42–51, fixative, Champy-Kull's; stain, Altmann's. Figure 52, fixative, Merkel's; stain, Flemming's triple, walls in spermatogenous tissue not stained. Figures 53–63, fixative, Regaud's; stain, Heidenhain's haematoxylin.

General Explanation of Plates

All prothallia were cut parallel to the axis.
All figures were drawn with the aid of the Abbé camera lucida (Zeiss).

Magnifications (of figures as printed)

Figures 4–10 = × 450
Figures 11–13, 22 and 23 = × 650.

Figures 14–19 = × 270.
Figure 20 = × 170.
Figure 21 = × 90.
Figures 24–63 = × 2000

PLATE 23

Fig. 1. Antheridial plant in which the notch is formed.
Fig. 2. Male prothallium: antheridia cover whole surface except the three anterior rows of cells; some spermatozoids discharged.
Fig. 3. Culture of prothallia of *Polypodium polypodioides* including larger archegonial plants and smaller antheridial ones.

PLATE 24

Fig. 4. Vertical section of antheridium initial showing first mitosis.
Fig. 5. Three-celled archegonium.
Fig. 6. Young archegonium with two neck cells.
Fig. 7. First mitosis of central cell of archegonium.
Fig. 8. Ventral cell of archegonium in mitosis.
Fig. 9. Mature archegonium showing wall separating the neck canal cells; egg indented.
Fig. 10. Mature archegonium showing shallow egg cell: ventral cell; and two neck canal cell nuclei.
Fig. 11. Spermatozoid entering egg.
Fig. 12. Spermatozoid within the egg nucleus.
Fig. 13. Spermatozoid nucleus within egg nucleus, chromatin of former becoming reticulate.
Fig. 14. One-celled embryo.
Fig. 15. Two-celled embryo: wall slightly oblique to neck of archegonium.
Fig. 16. Four-celled embryo.
Fig. 17. Young embryo.
Fig. 18. Young embryo.
Fig. 19. Embryo showing primary organs differentiated; octant walls still distinct.
Fig. 20. Embryo slightly older than in fig. 19; octant wall distinct, but irregular.
Fig. 21. Older embryo: cotyledon initial; region of stem-growing point; foot; region of root-growing point.

PLATE 25

Fig. 22. Antheridium showing upper and lower wall cells and central cell.
Fig. 23. Antheridium with upper and lower wall cells, central cell and cap cell.
Fig. 24. Spermatid mother cell: two blepharoplasts.
Fig. 25. Spermatid mother cell in prophase: two blepharoplasts.
Fig. 26. Spermatid: blepharoplast in cytoplasm at some distance from nucleus.
Fig. 27. Spermatid: blepharoplast applied to nuclear membrane.
Fig. 28. Spermatid: blepharoplast beginning to elongate.
Fig. 29. Spermatid: nucleus beginning to condense; blepharoplast more than half around nucleus.
Fig. 30. Crescent shaped spermatid nucleus.
Fig. 31. Coiled spermatozoid: nucleus condensing; blepharoplast extending beyond nucleus.

Fig. 32. Spermatozoid found in neck of archegonium: 2.5 coils; cilia distinct.
Fig. 33. One-celled antheridium: central cell with two large irregular nucleoli.
Fig. 34. Two-celled antheridium: one nucleus with two nucleoli; one with constricting nucleolus.
Fig. 35. Four-celled antheridium: three nucleoli in section; two nuclei with one nucleolus each; one nucleus with constricting nucleolus.
Fig. 36. Eight-celled antheridium: four cells in section; three nuclei with two nucleoli; one nucleus with constricting nucleolus.
Fig. 37. Spermatid: blepharoplast near nucleus; two nucleoli.
Fig. 38. Spermatid: blepharoplast applied to nuclear membrane, one nucleolus.
Fig. 39. Spermatid: blepharoplast applied to nuclear membrane; two nucleoli.
Fig. 40. Spermatid: blepharoplast elongated; one nucleolus.
Fig. 41. Spermatid: blepharoplast elongated; two nucleoli.

PLATE 26

Fig. 42. Prothallial cell dividing to form anteridium initial: large plastids; mitochondria near poles of spindle.
Fig. 43. Antheridium showing central cell and two wall cells; plastids in wall cells large; plastids and mitochondria in the central cell.
Fig. 44. Two-celled antheridium: plastids in wall cells; plastids and mitochondria in spermatogenous tissue.
Fig. 45. Four-celled antheridium: plastids and mitochondria.
Fig. 46. Spermatid mother cell at metaphase: blepharoplasts at poles of spindle.
Fig. 47. Spermatid—blepharoplast applied to nuclear membrane; mitochondria in cytoplasm.
Fig. 48. Spermatid: nucleus beginning to flatten; blepharoplast half way around nucleus; mitochondria in cytoplasm.
Fig. 49. Spermatid: part of coiling nucleus; mitochondria on surface of nucleus and in nearby regions of cytoplasm.
Fig. 50. Mature spermatozoid: blepharoplast applied to anterior portion of nucleus and extending .5 of a coil beyond it; mitochondrial granules along inner surface of blepharoplast and nucleus.
Fig. 51. Mature spermatozoid: blepharoplast applied to anterior portion of nucleus and extending .5 of a coil beyond it; mitochondria as two rows along inner surface of nucleus and one row along blepharoplast.
Fig. 52. Mature spermatozoid with starch in protoplasmic vesicle.
Fig. 53. Antheridium initial: mitochondria in broken network.
Fig. 54. Spermatid: mitochondria as short rods.
Fig. 55. Spermatid: mitochondria as short and long rods.
Fig. 56. Spermatid: mitochondria as short rods; plastids.
Fig. 57. Spermatid: mitochondria as short and long rods.
Fig. 58. Spermatid: mitochondria forming broken network.
Fig. 59. Spermatid nucleus beginning to coil; mitochondria surrounding nucleus.
Fig. 60. Spermatid: nucleus coiling; mitochondria around nucleus.
Fig. 61. Mature spermatozoid: two rows of mitochondria along inner surface of nucleus; one row extending beyond it.
Fig. 62. Mature spermatozoid showing two rows of mitochondria.
Fig. 63. Mature spermatozoid showing two rows of mitochondria.

PLATE 23

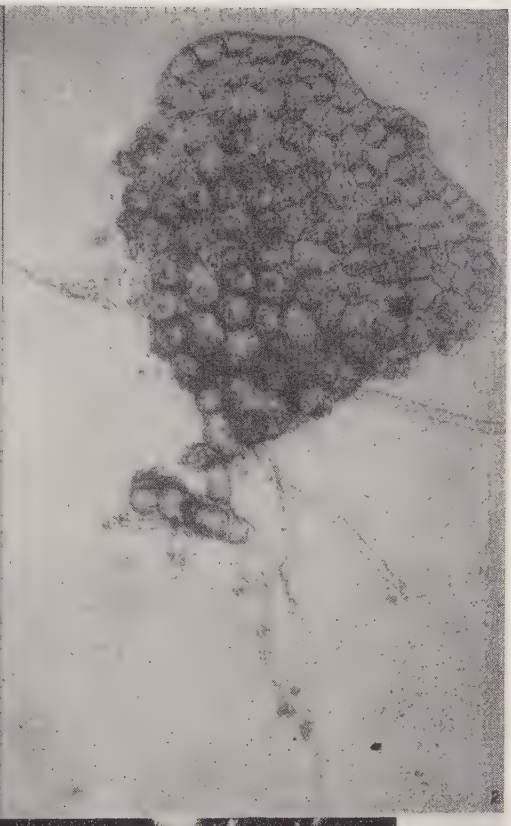


PLATE 24

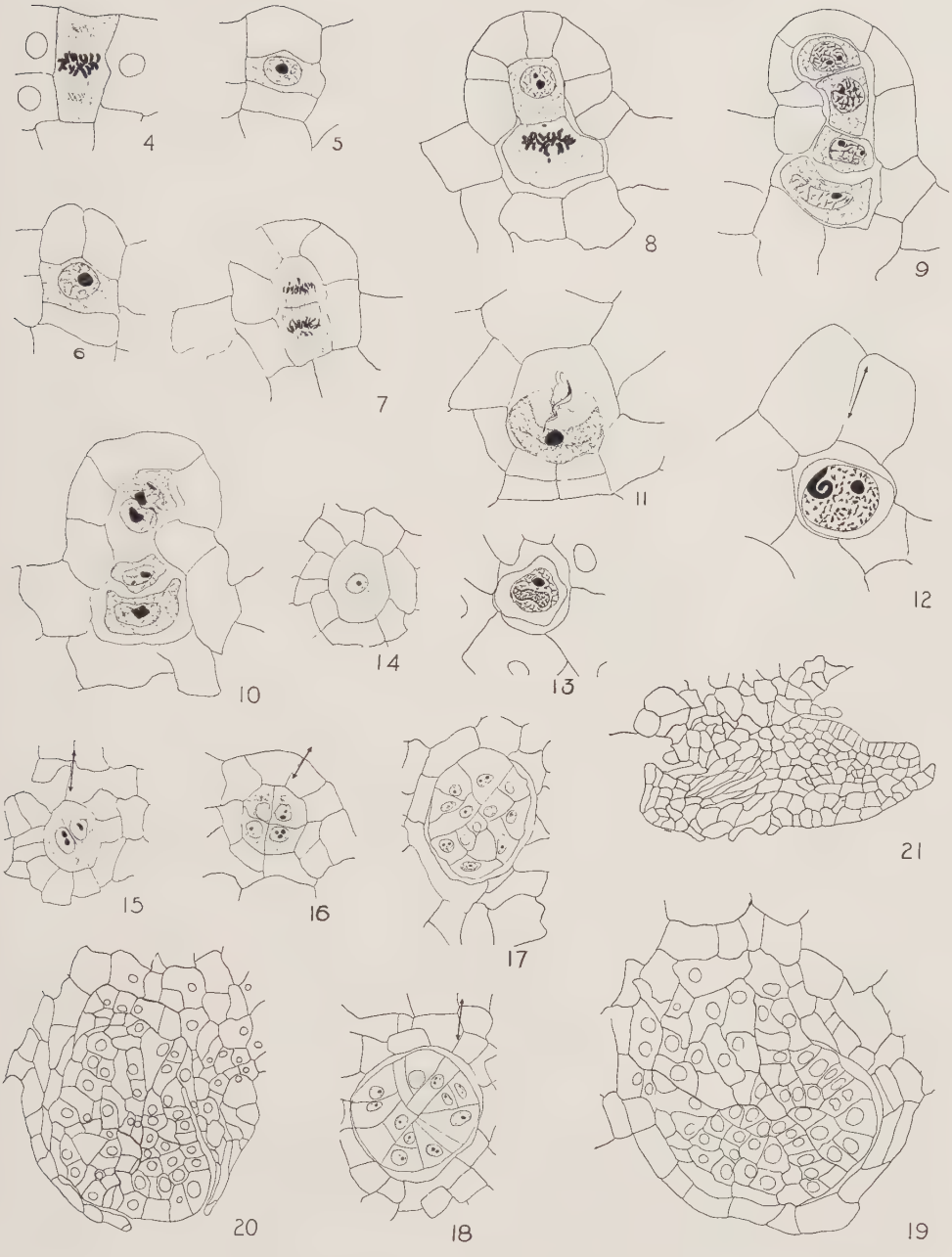
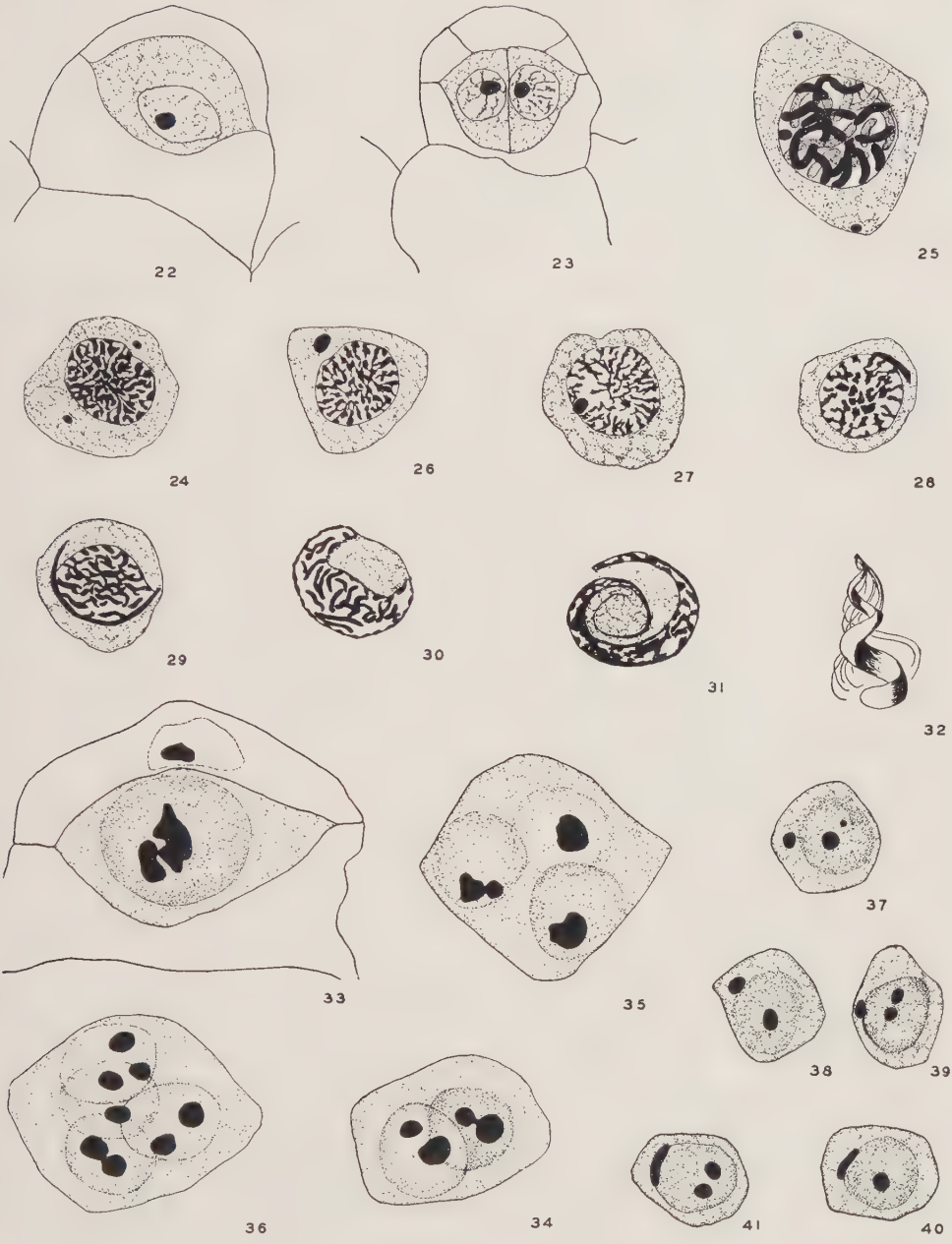


PLATE 25



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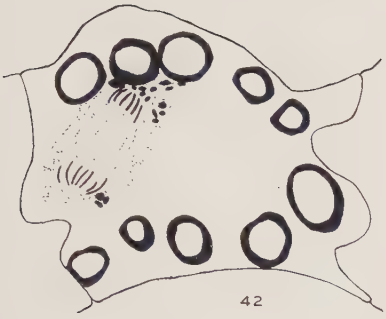
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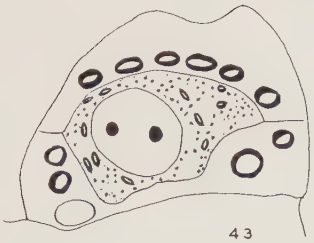
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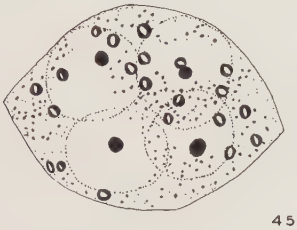
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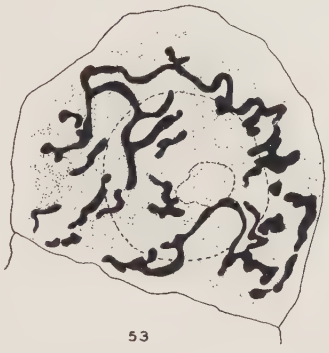
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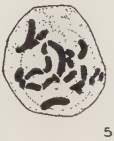
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VITA

Douglass Evans Rankin, born in Fayetteville, North Carolina, January 31, 1905, received her early education in the public schools of that city, graduating from the Fayetteville High School in 1923. In the fall of 1923 she entered Agnes Scott College in Decatur, Georgia, from which institution she received an A.B. degree in 1927.

During the school year of 1927–1928 she was principal of a grammar school near Dunn, North Carolina.

In October 1928 she began graduate work in the Botany Department of the Johns Hopkins University. She worked as part-time assistant to Dr. Ralph E. Cleland, Goucher College, during the years 1930–1932. The summer of 1930 was spent in studying marine algae at Woods Hole, Massachusetts. During the summer of 1932 she was a member of the Johns Hopkins Jamaican Expedition, organized for the purpose of studying tropical botany.

She is an associate member of the Johns Hopkins chapter of Sigma Xi.